

U.S.S.N. 7/544,045
 OMRF 178

1636

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NOV 01 2000

TECH CENTER 1500/2900

loxP vs. loxK1 vs. loxK2

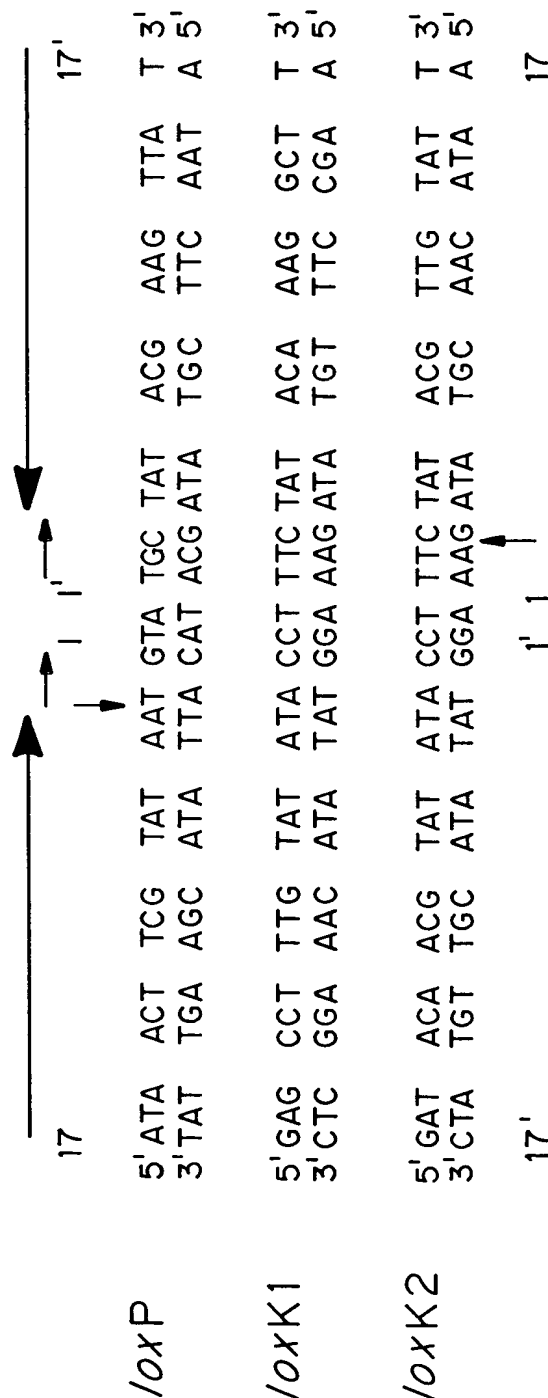


FIG. 1

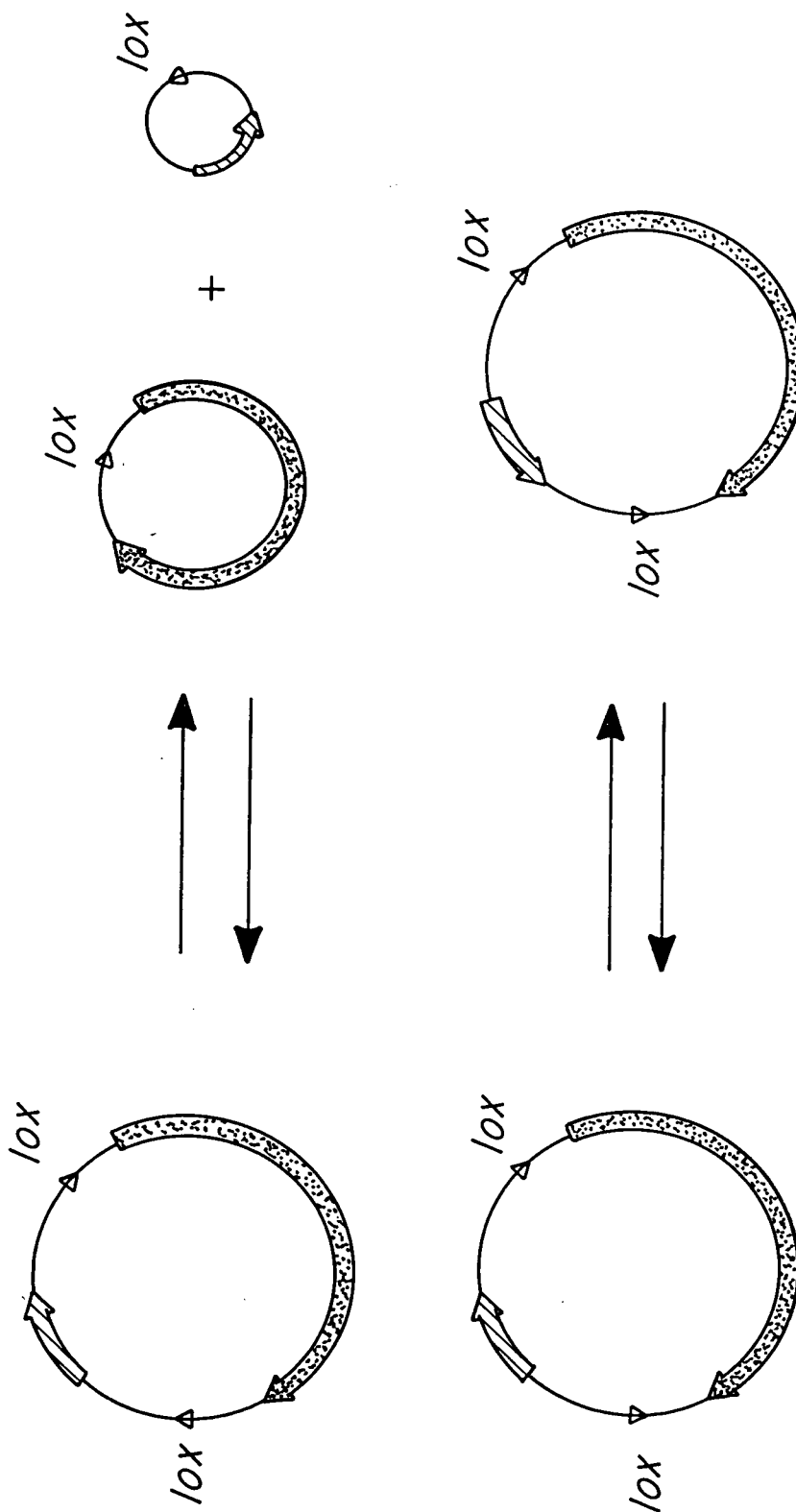


FIG. 2

BY	FIG.	
DRAFTSMAN	CLASS	SUBCLASS

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TECH CENTER 1600/2900

FIG. 3

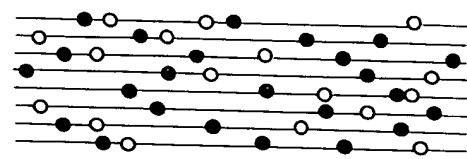
cre

PCR AMPLIFICATION

MUTAGENIC:

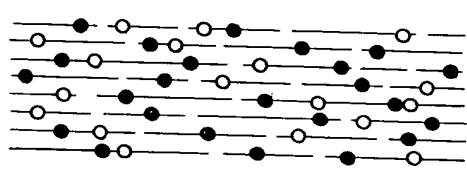
- BENEFICIAL MUTATION
- DELETERIOUS MUTATION

Pool of related sequences



DNASE I DIGEST

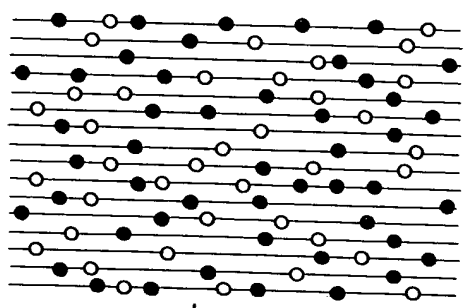
Pool of random size fragments



REASSEMBLY BY PCR

in vitro RECOMBINATION PLUS MUTAGENIC EVENT

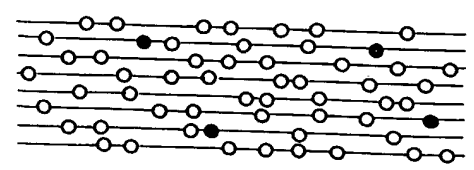
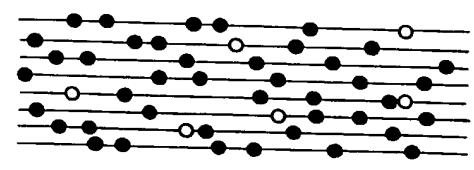
Large pool of recombined mutants



SELECTION

CLONES WITH NEGATIVELY CONTRIBUTING MUTATIONS ARE ELIMINATED

COMBINATIONS OF POSITIVE MUTATIONS STAY IN THE POOL



REPEAT

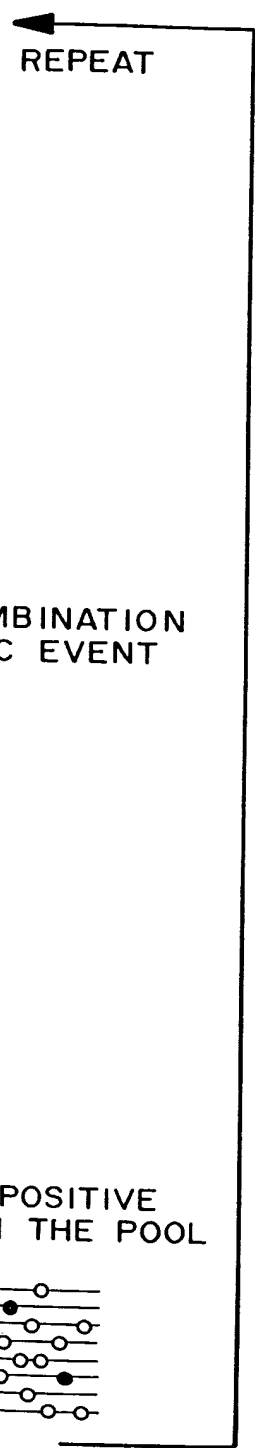


FIG. 4

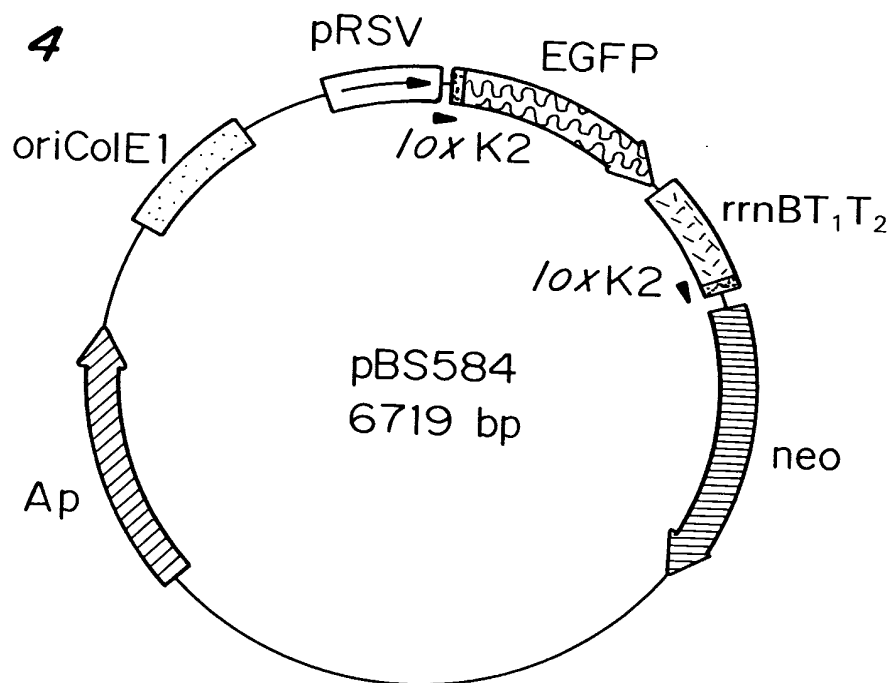


FIG. 6

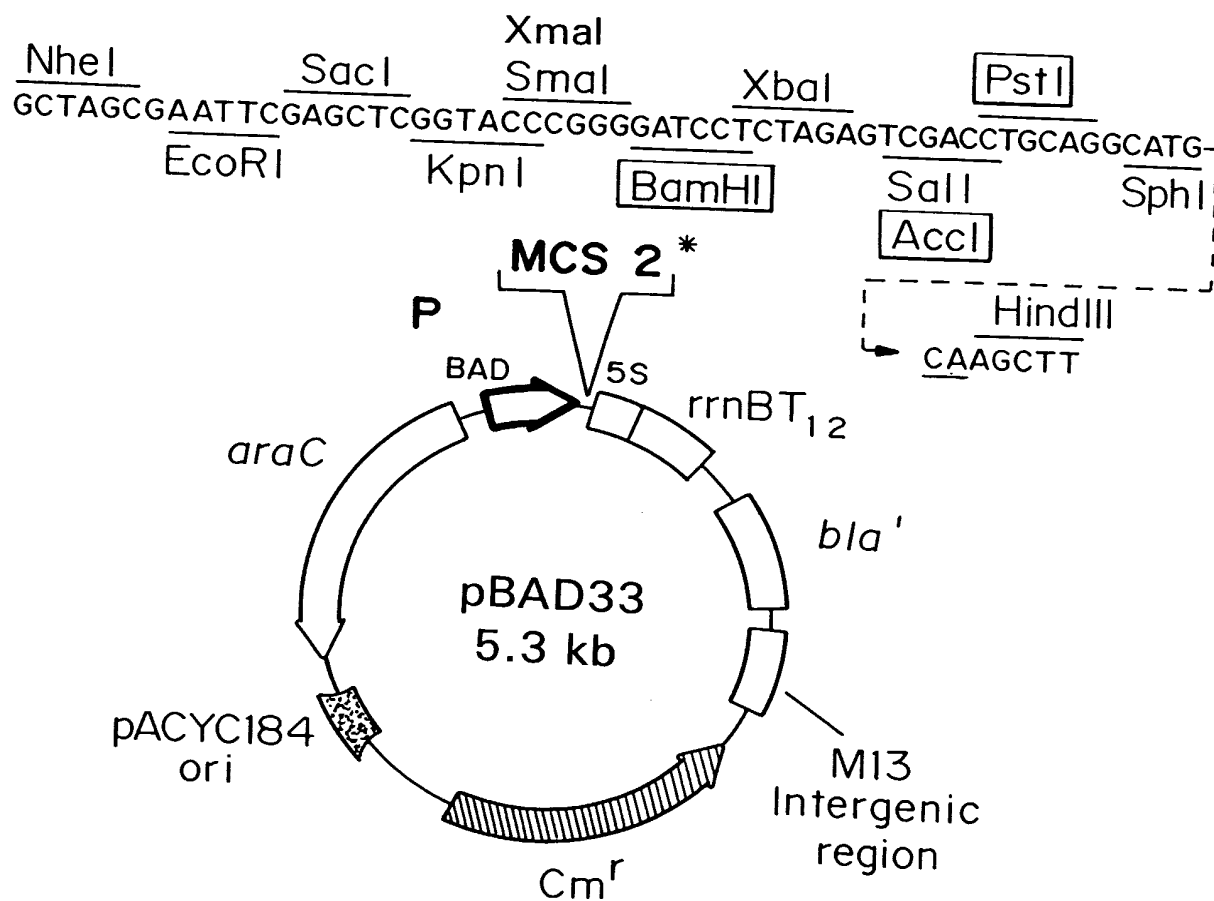


FIG. 5A

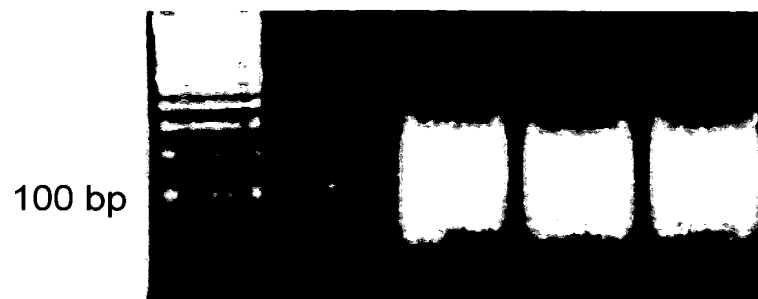


FIG. 5B

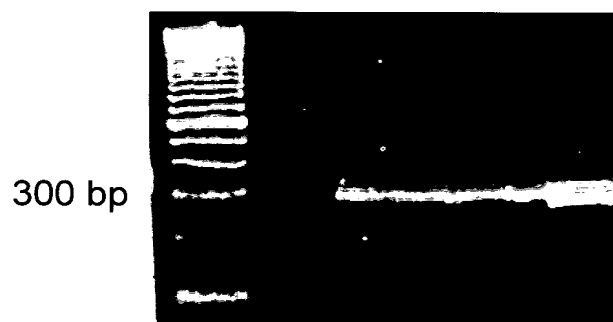
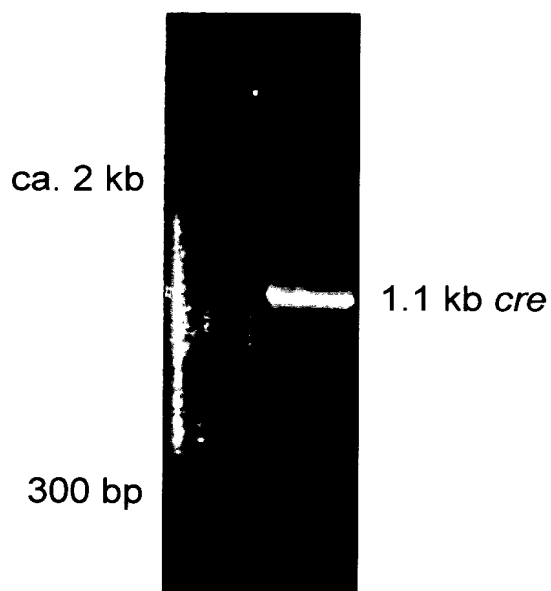
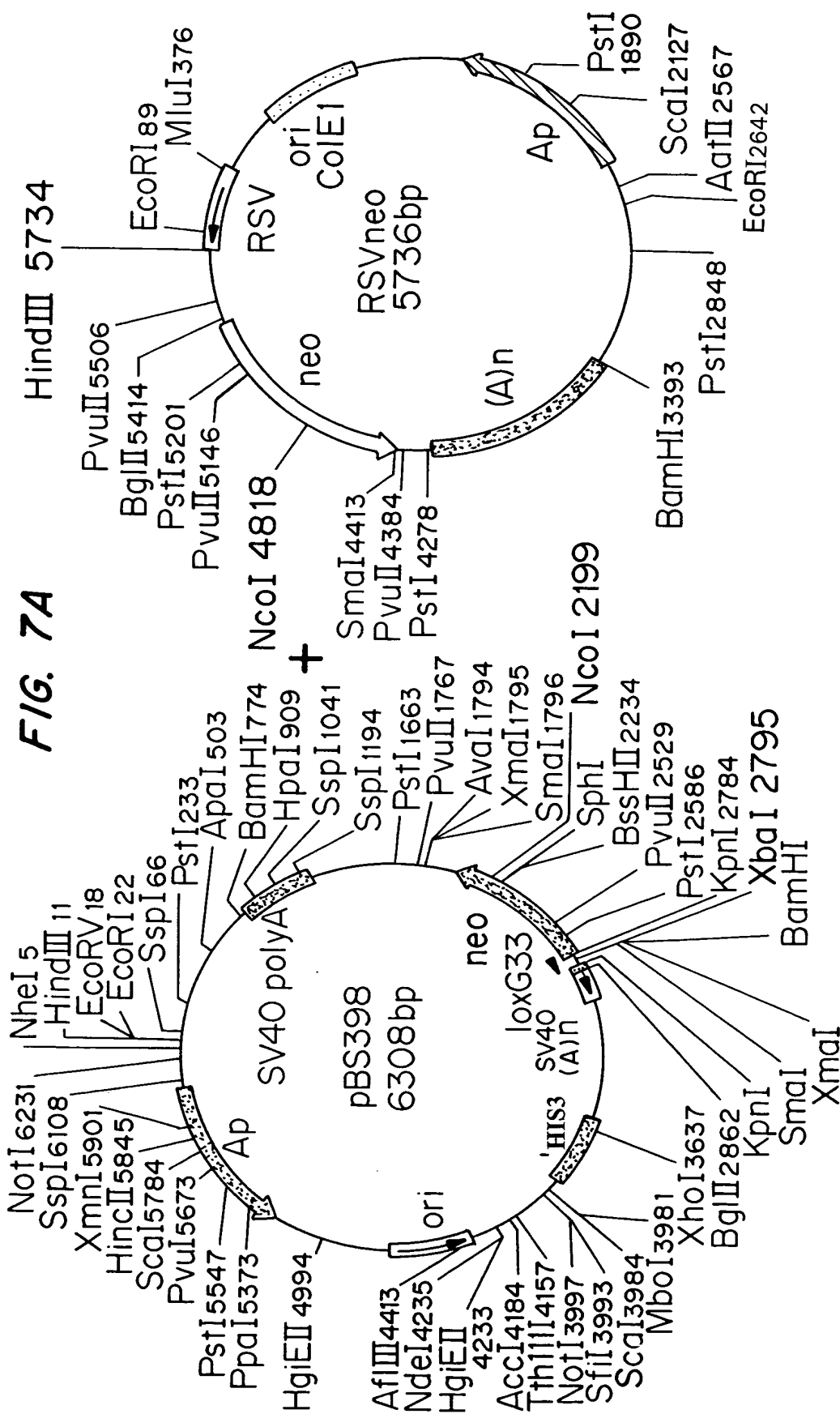


FIG. 5C





CONTINUED ON FIG. 7B

MCS for pBS 561-2 sets of oligonucleotides

<i>HindIII</i>		<u>SD</u>		Start		<i>SalI</i>		<i>NheI</i>		<i>BglII</i>	
5'	AGC	TTG	GAG	GCT	ATC	ATG	TCG	ACC	CTA	GCA	
3'	AC	CTC	CTG	CGA	TAG	TAC	AGC	TGG	GAT	CGT	
<i>BglII</i>		<i>EcoRV</i>		<i>NotI</i>		Stop		<i>XhoI</i>		<i>XbaI</i>	
5'	GAT	CTG	ATA	TCT	GCG	GCC	GCT	GAC	ACT	CGA	GT
3'	AC	TAT	AGA	CGC	CGG	CGG	CGA	CTG	TGA	GCT	CAG
											ATC
											5'

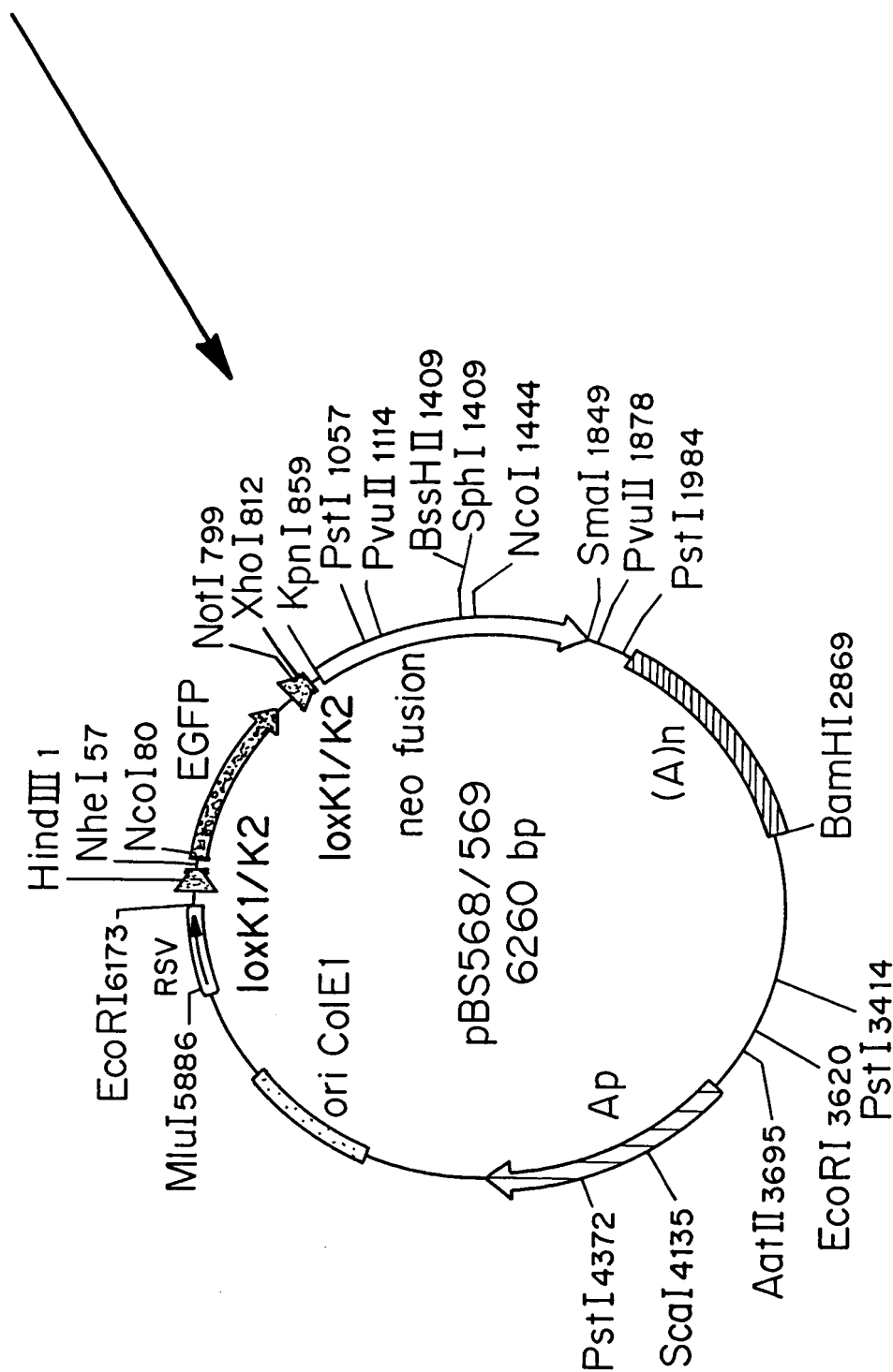
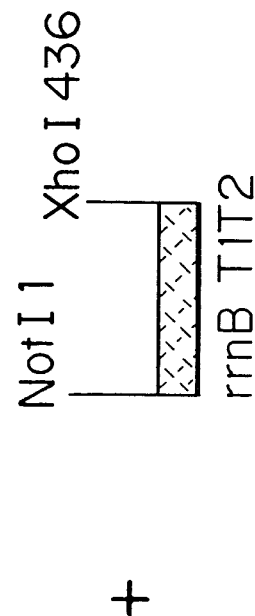
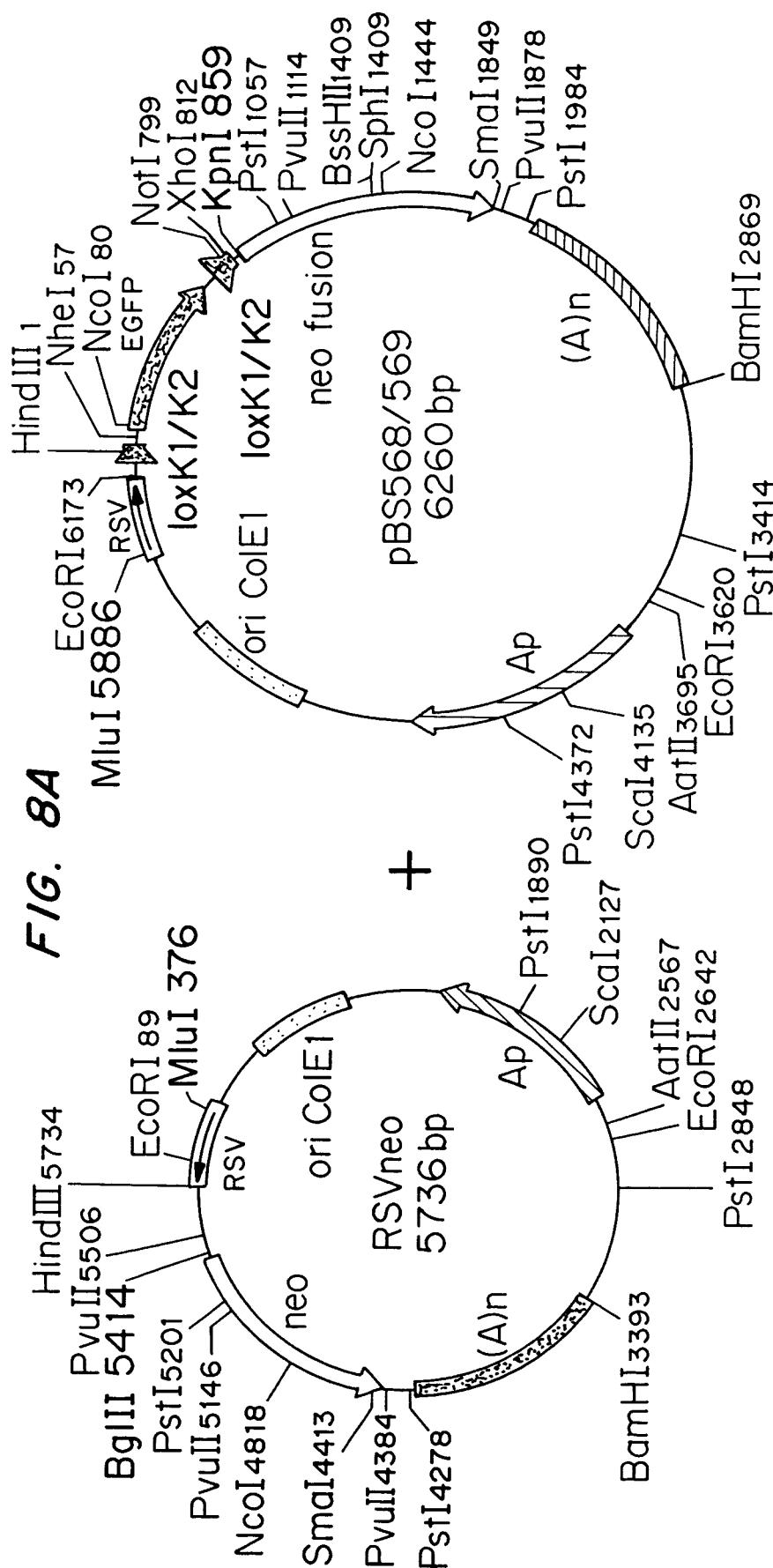


FIG. 7C

FIG. 8A



CONTINUED ON FIG. 8B

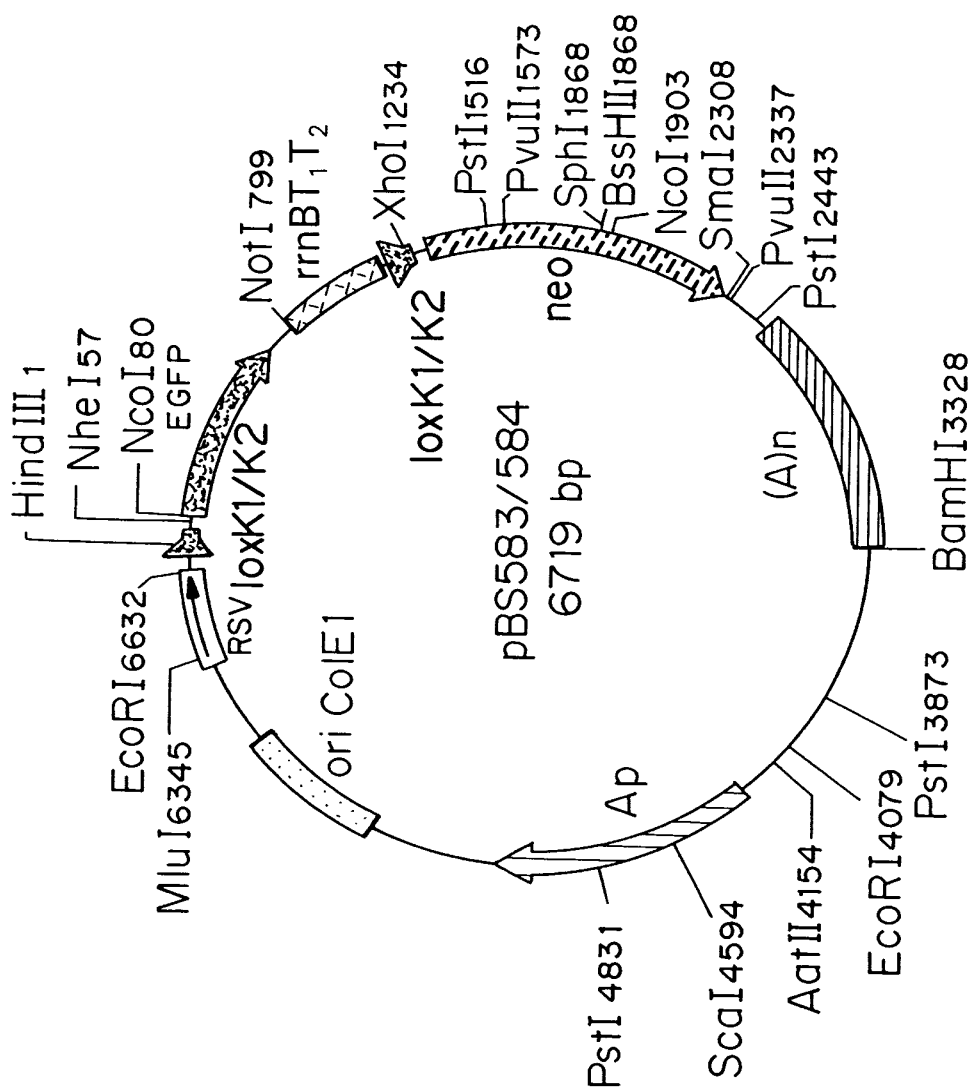


FIG. 8B

FIG. 9

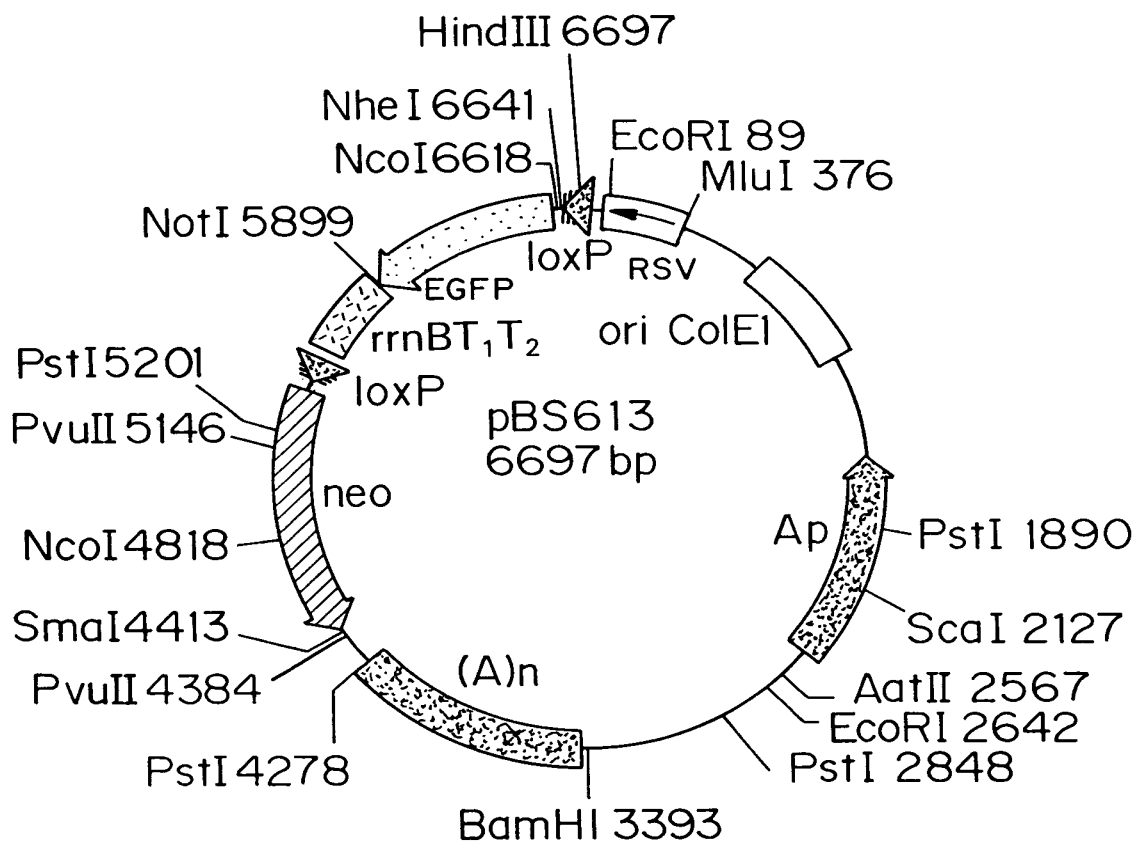
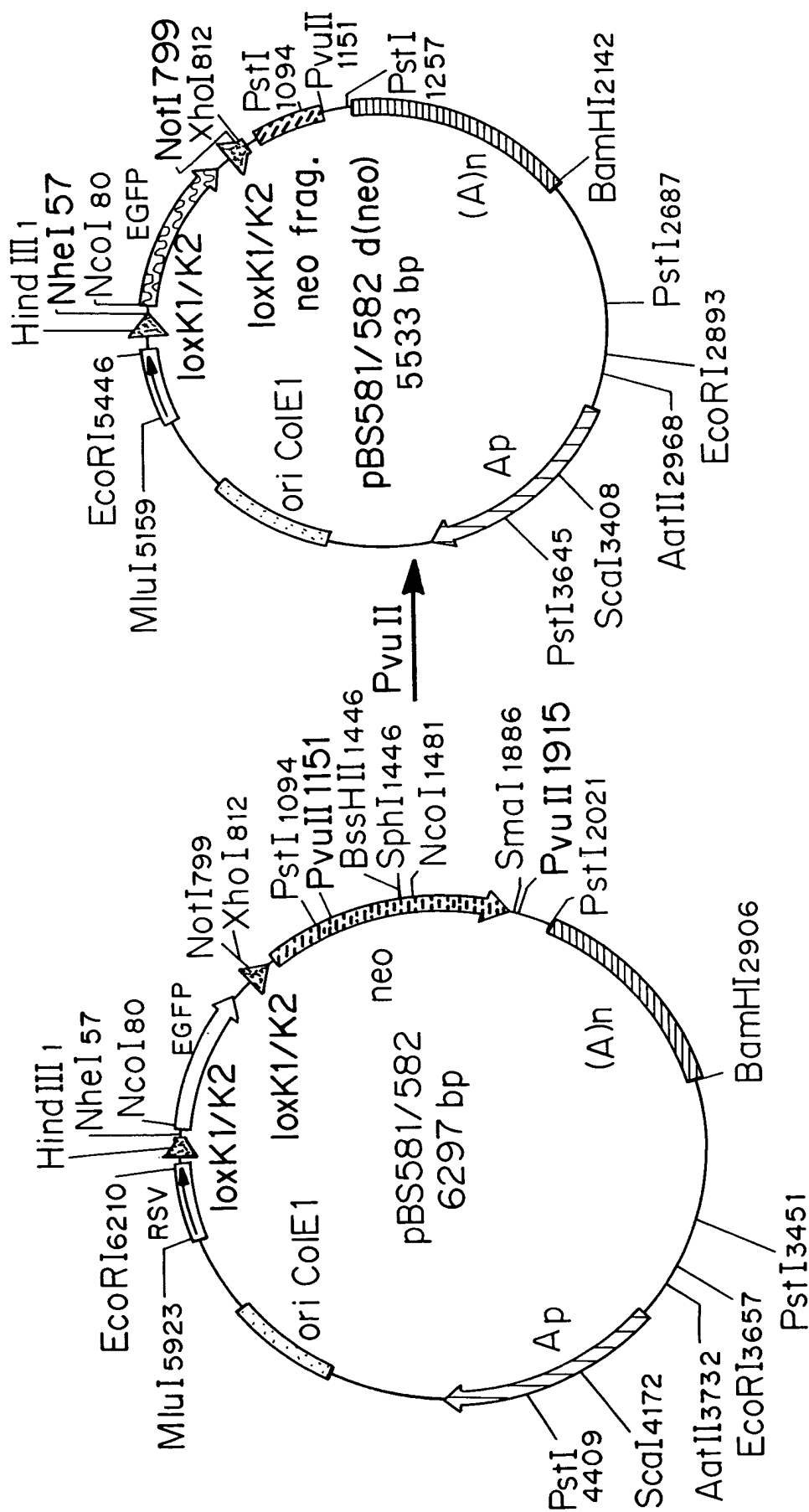
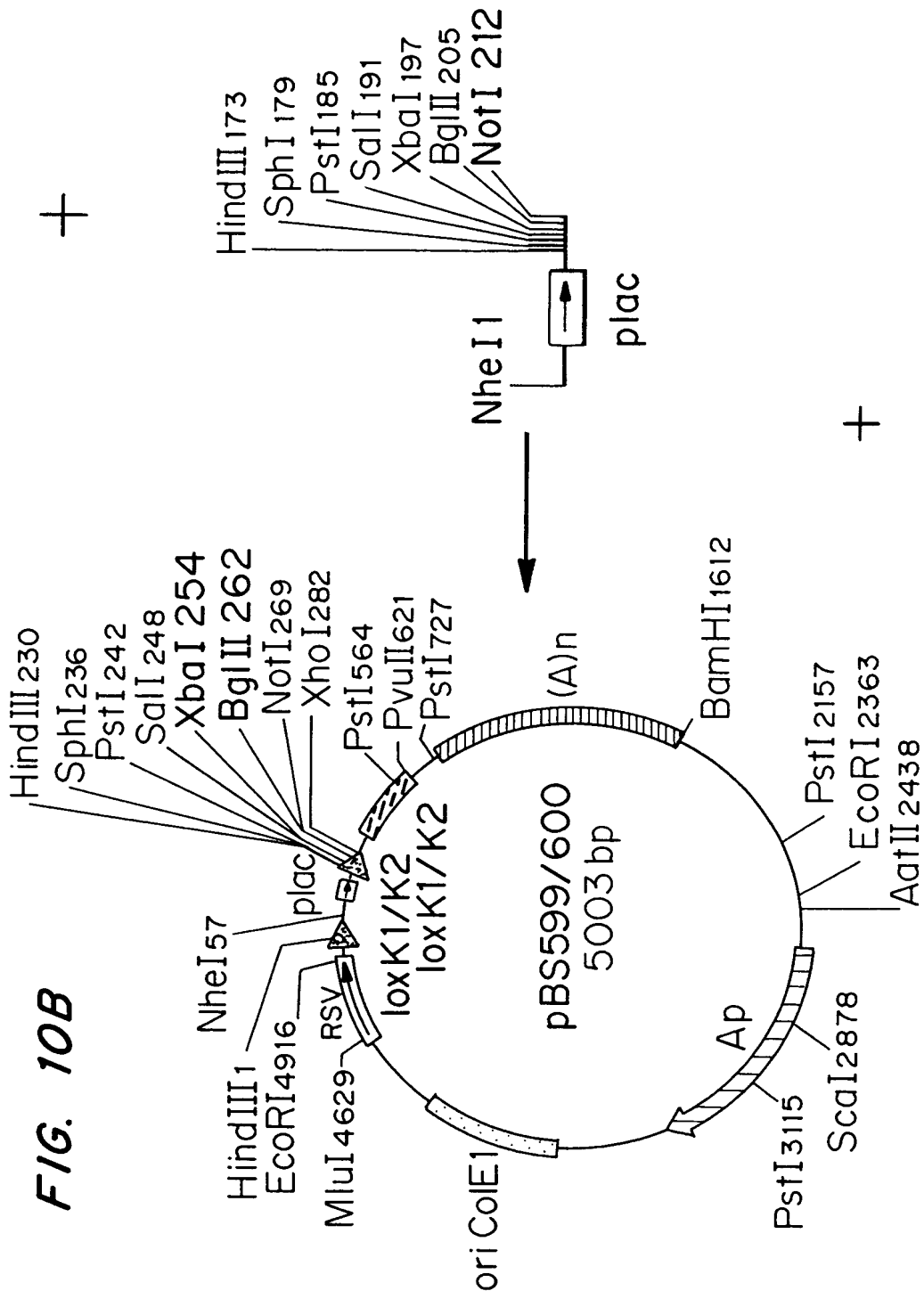


FIG. 10A



CONTINUED ON FIG. 10B



CONTINUED ON FIG. 10C

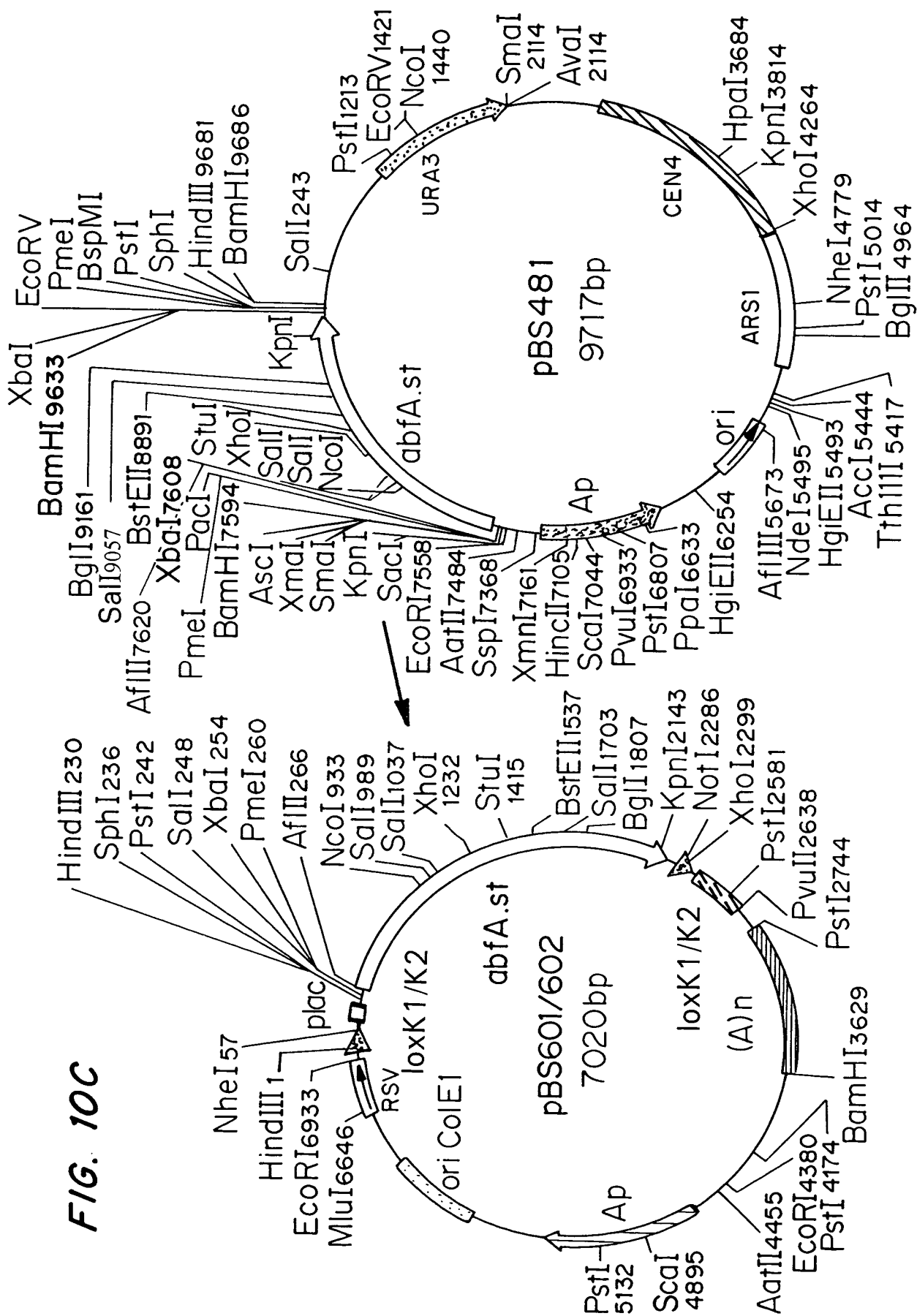
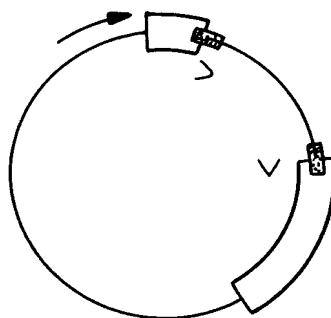


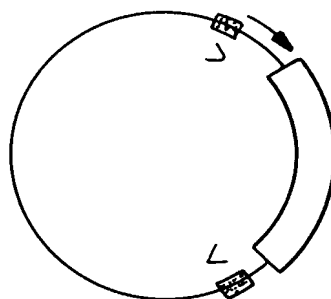
FIG. 10C

FIG. 11

INTERRUPTED
(DELETION)



FLANKED
(DELETION)



INVERTED
(INVERSION)

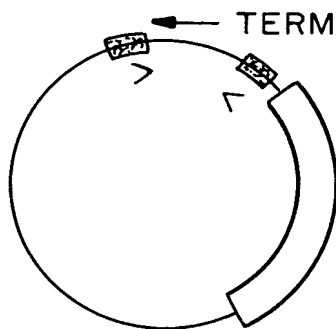
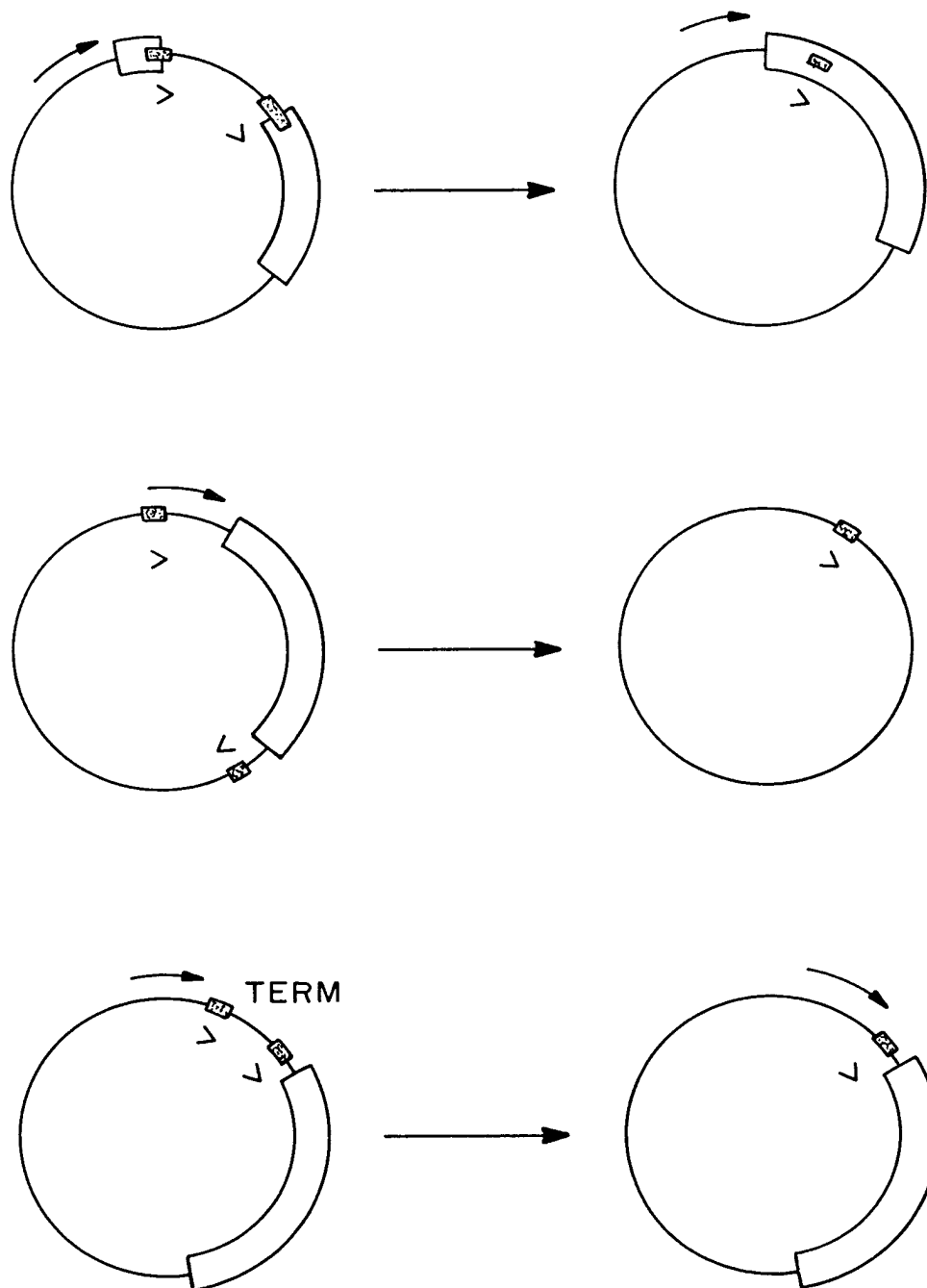


FIG. 12A

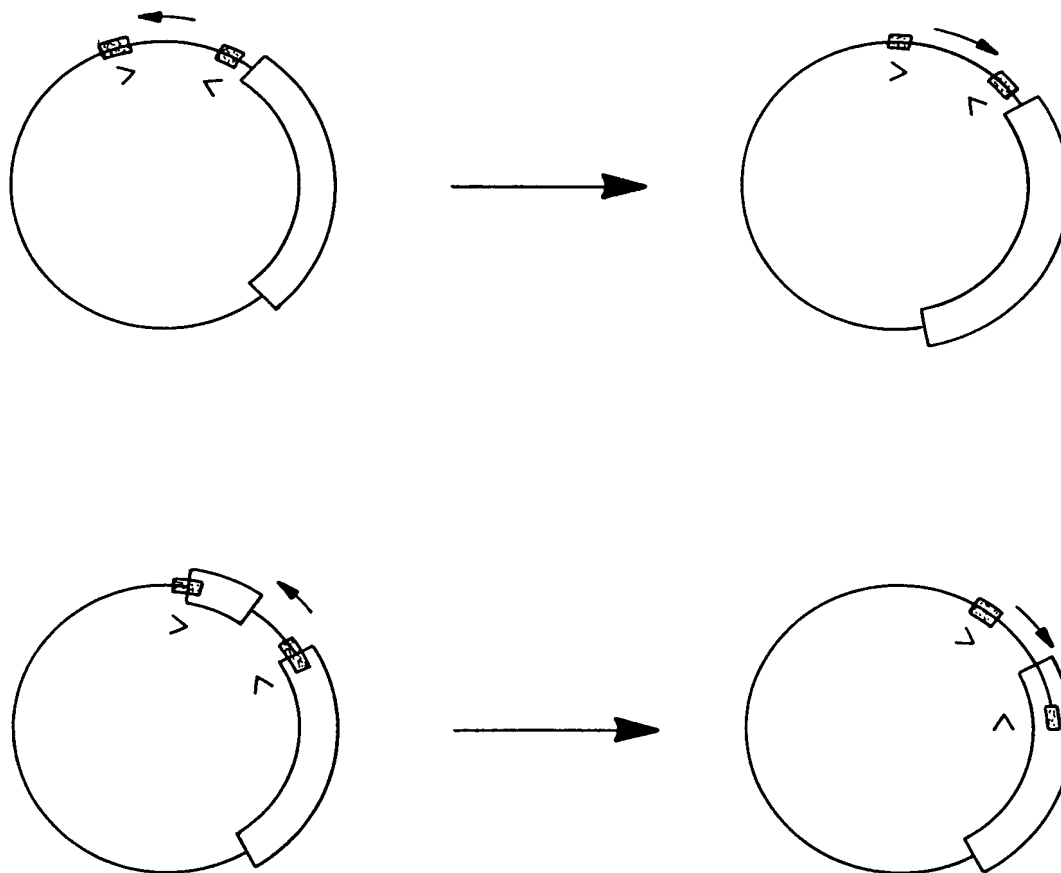


DELETION

FIG. 719.		
CLASS	SUBCLASS	
DRAWING		

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FIG. 12B



INVERSION

FIG. 12C

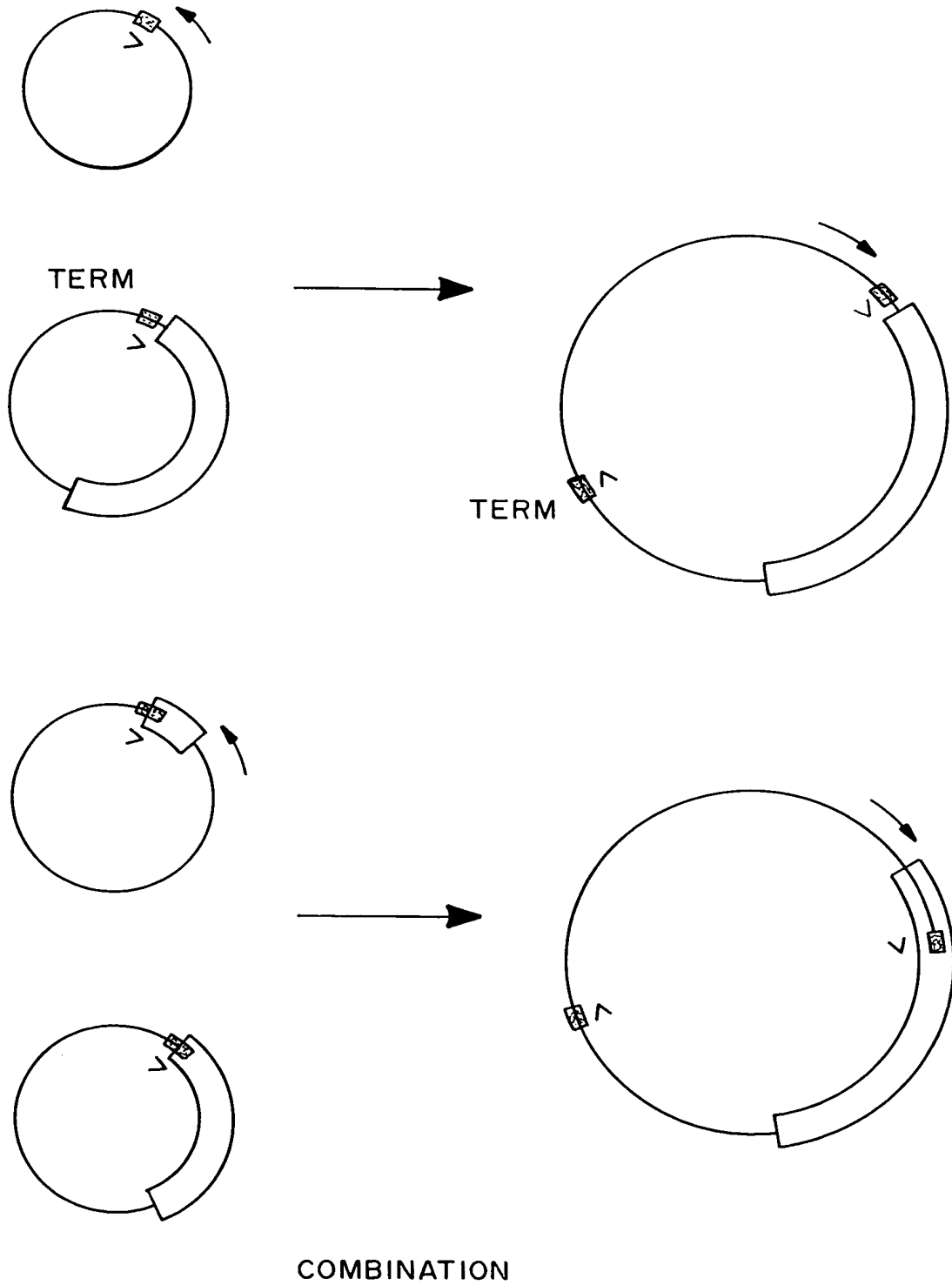
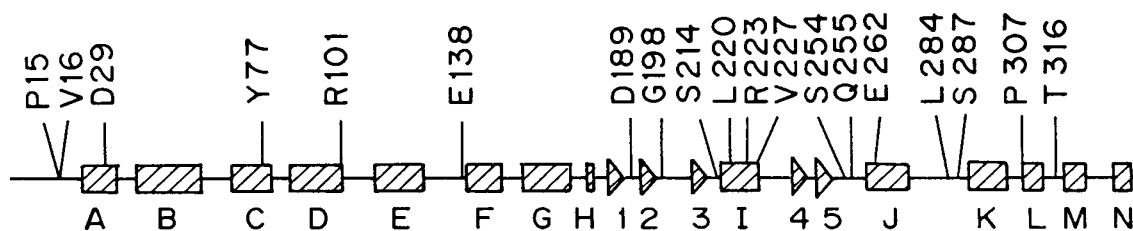


FIG. 13



R3M1	P - A	-	-	-	N - S - - I - - G - - -
R3M2	P - -	-	-	G	- - - - - G - G - S - S
R3M3	- I -	-	-	-	N S - - Q - - R G - S L -
R3M4	- - -	C	-	K	- - - - - - - - Q - - -
R3M5	- - A	-	Q	-	- - - Q - - - G - - - S
R3M6	- - -	-	-	-	- - - - - - - G Q - - S

FIG. 14A

% of Cre - mediated recombination

R3M3 Cre	97	82	67	90
E262G/D29A Cre	95	37	34	47
E262G/T316S Cre	98	21	14	32
E262G/D189N Cre	93	6	5	22
E262G Cre	90	20	10	28
wt Cre	95	0	0	0
	TT	GG	CC	AA

loxP Halfsite ATA A C T T C G T A T A

FIG. 14B

% of Cre-mediated recombination

R3M3 Cre	97	90	95	30	82
E262G/D29A Cre	95	47	80	2	30
E262G/T316S Cre	98	32	81	1	12
E262G/D189N Cre	93	22	71	1	17
E262G Cre	90	28	78	2	20
wt Cre	95	0	18	0	5
	<i>loxP</i> ²	<i>loxK</i> ²	<i>loxP-loxK</i> ²	<i>loxP-loxK</i> ¹	<i>loxK</i> ¹

FIG. 15

% Cre-mediated lox^2 recombination

R3M3 Cre	97	99	92	82	56	67	90
E262G/D29A Cre	95	95	82	37	35	34	47
E262G/T316S Cre	98	97	89	21	31	14	32
E262G/D189N Cre	93	98	68	6	9	5	22
E262G Cre	90	97	65	20	32	10	28
wt Cre	95	92	25	0	0	0	0

TT TG GT GG TC CC AA

$loxP$ Halfsite ATAACCTTCGTATA

lox Type	Ligand	% Binding (approx.)
P	wt	100.0
	R3M3	100.0
K2	wt	100.0
	R3M3	100.0
	G	40.0
	GA	38.0
	GN	42.0
K1	wt	100.0
	R3M3	100.0
	G	18.0
	GA	15.0
	GN	18.0

wt FRT: GAAGTTCCTATTC TCTAGAA GTATAGGAACTTC

Note - alternatively, a full site can also be used having 3 repeat elements:

GAAGTTCCTATTC CCGAAGTTCCTATTC TCTAGAA GTATAGGAACTTC

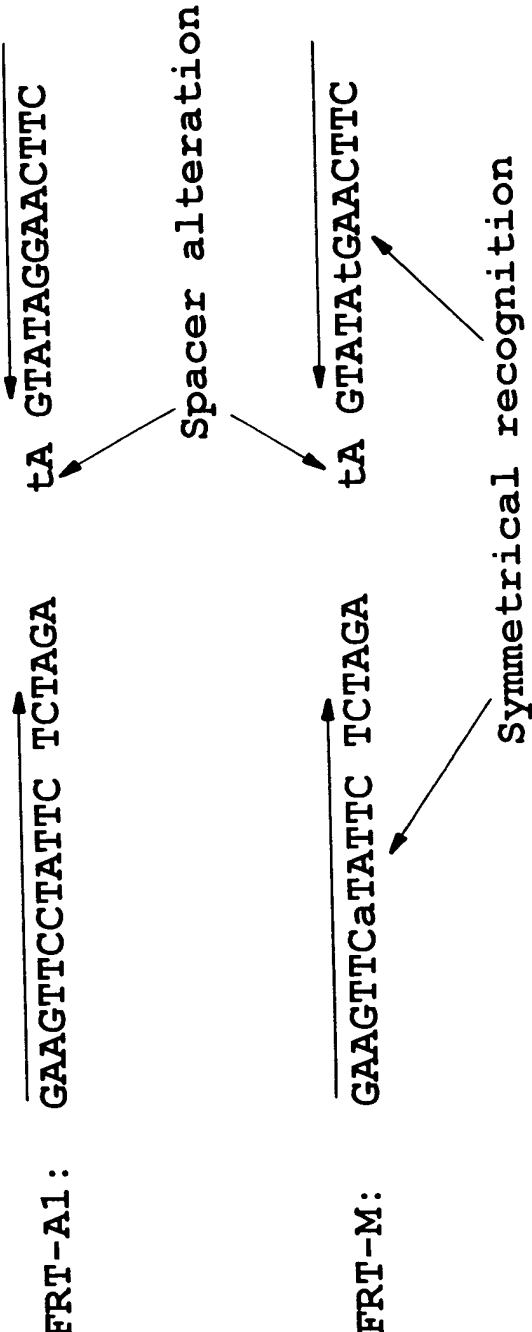
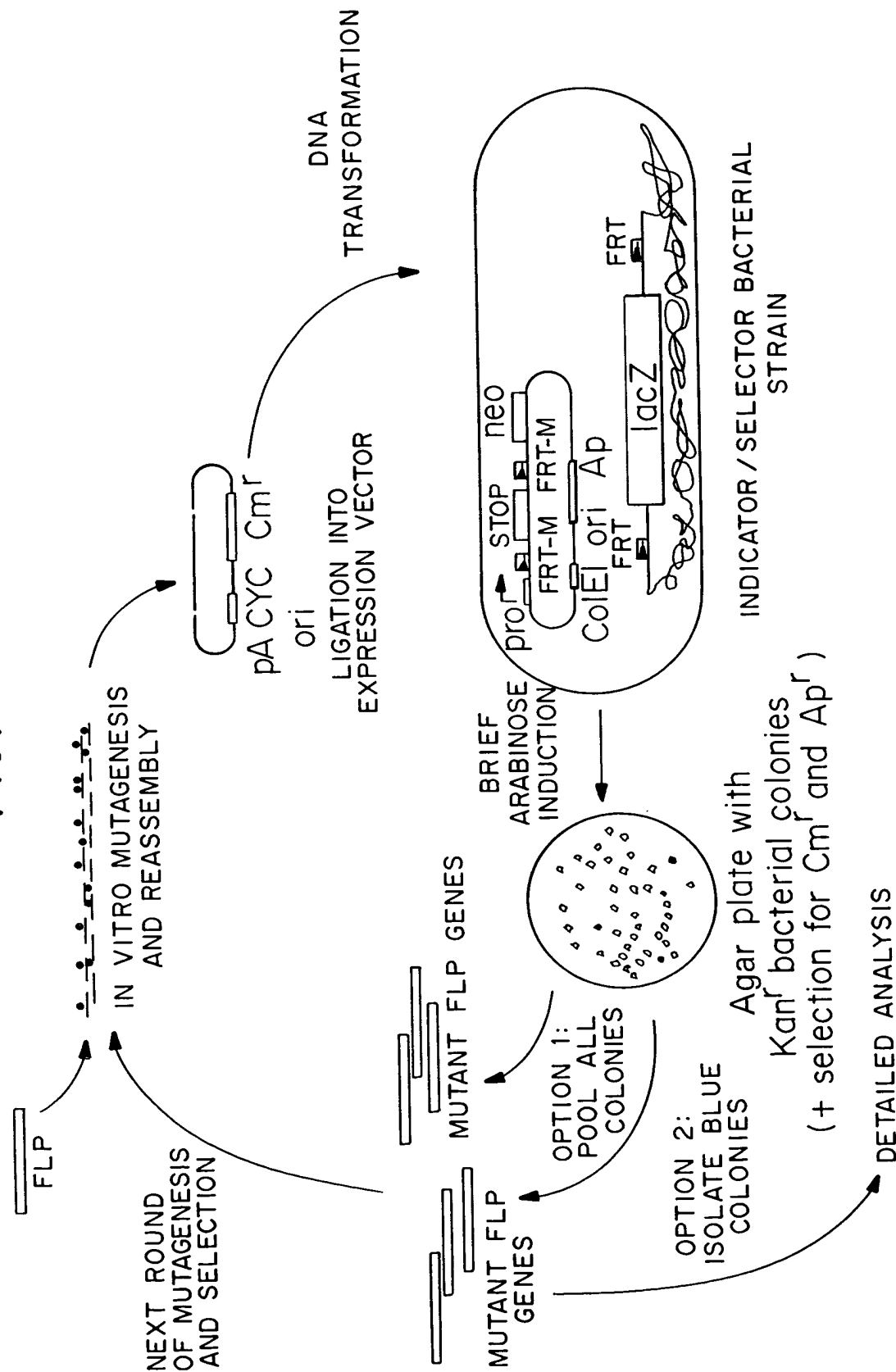


FIG. 17

FIG. 18



wt FRT: GAAGTTCCTATTC TCTAGAAA GTATAGGAACTTC
 Note - alternatively, a full site can also be used
 having 3 repeat elements:
GAAGTTCCTATTCCGAAGTTCCTATTC TCTAGAAA GTATAGGAACTTC

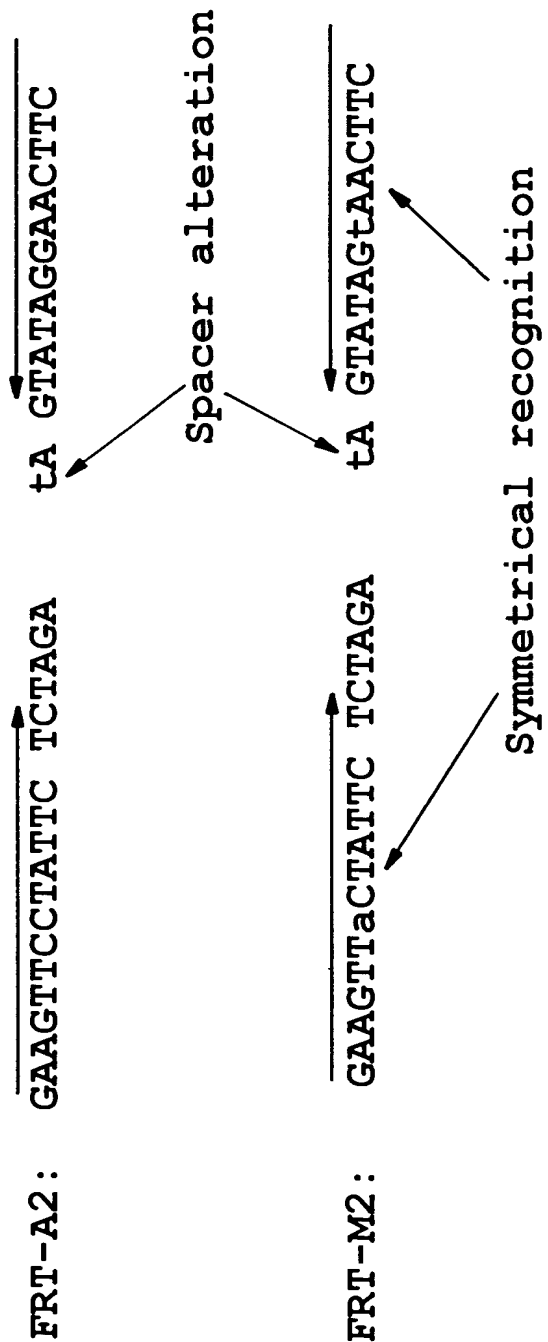


FIG. 19